PAPERS.

8. Observations on the Cytology of Flagellates and Amœbæ obtained from old Stored Soil. By T. Goodey, D.Sc.*, Protozoologist, Research Laboratory in Agricultural Zoology, University of Birmingham.

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(Plates I.–IV. and Text-figure 1.)

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Introduction.

Within the last few years a good deal of attention has been paid to soil-protozoa, owing to the important function ascribed to them by Russell and Hutchinson† in their hypothesis advanced to account for the changes observed on partially sterilising soil. According to these investigators, soil-protozoa act as a factor limiting bacterial activity, and so prevent a normal soil from attaining its full fertility.

It is of importance, therefore, that we should ascertain what kinds of protozoa are present in the soil and as much as possible

* Communicated by Prof. F. W. Gamble, F.R.S., F.Z.S.

† Russell and Hutchinson, Journ. Agric. Sci. vol. iii. pt. xi. (1909), and vol. v. pt. xi. (1913).

of their life-histories; also whether they are forms capable of consuming bacteria, and thus able to function as a limiting factor on soil-bacteria.

One of the chief methods by which soil-protozoa can be studied is examination in cultures made in suitable media; and although it has recently been claimed * that the cultural forms are not necessarily those occurring in a trophic condition in the soil, and may not therefore be concerned in the biological changes of the soil, yet I have quite recently obtained some experimental results which point most positively to the cultural protozoa, especially amœbæ, acting as a check to the increase of bacterial numbers. I hope to publish an account of this work shortly. In the present paper an account is given of a few forms of flagellates and amœbæ which were obtained culturally from some old soils stored at Rothamsted Experimental Station, together with some observations on their cytology and methods of division. The protozoan fauna of these soils was a limited one when compared with that of an ordinary garden or field-soil, and for this reason presented a suitable field for working out the different organisms in detail.

In a recent number of 'The Annals of Applied Biology' I have recorded the culture of amœbæ and flagellates from soil bottled so far back as 1865 and left untouched since then; thus proving the survival of protozoa, no doubt in an encysted condition, for a period of 49 years. It was my intention at the outset of the work merely to obtain an idea of the character of the protozoan fauna surviving in the different soils examined. In order to do this, and to determine as nearly as possible the different species which cropped up, it was necessary to make a number of permanent stained preparations and to study these in considerable detail. As a result of these observations, I have obtained a number of interesting facts on the cytology, mode of nuclear division, etc., in several of the forms examined.

The samples of soil tested for protozoa were taken from bottles of soil obtained originally from five of the fields under experimental cultivation at Rothamsted. These were: Broadbalk soil bottled in 1865, Geescroft soil bottled in 1865, Agdell soil bottled in 1867, Hoosfield soil bottled in 1868, and Barnfield soil bottled in 1870.

Methods.

As a culture medium, saline egg-albumen was used and found very serviceable. A small quantity of soil was placed in this medium, contained in a circular flat-bottomed glass dish furnished with a close-fitting flat lid. This was then put into an incubator at 22° C., or left at room-temperature. After a few days, microscopic examination of the culture revealed active protozoa. These were frequently found on the surface or at the bottom of the

* Russell, E. J., "Soil Protozoa and Soil Bacteria," Proc. Roy. Soc. B, vol. lxxxix. p. 76 (1915).

liquid; and in order to obtain these forms, coverslips were floated on the surface and placed on the bottom. In other cases the vast majority of the organisms occurred about midway in the depth of the culture, and in order to obtain coverslip-preparations of these I drew out small quantities of the culture where the protozoa were thickest by means of a fine pipette, and then made smears with the liquid. As a rule, three cultures were made from each soil, in order to obtain a representative fauna, and the cultures were examined for several days to note any succession of forms. Coverslip-preparations were fixed in Maier's solution or in Bouin's fixative, and iron-hæmatoxylin was used as the stain throughout; occasionally preparations were counterstained with lichtgrün-picric.

I propose in the following pages to deal with the protozoa encountered under the heading of the group to which they belong, mentioning the particular soil or soils from which they were taken.

A. Flagellata.

(1) Prowazekia (Bodo) saltans Ehrbg. (Pl. I.)

In one of my cultures of Barnfield 1870 soil a very small jumping flagellate occurred in abundance at the bottom of the liquid. It appeared somewhat bean-shaped when seen under a low power, and I at once concluded that it was *Bodo saltans*. A coverslip-preparation was made, and on it I obtained many dividing organisms, from a study of which I have been able to work out fairly completely the process of nuclear division. I will describe the structure of a normal organism, and then deal with the question of identification and nomenclature.

(a) *Structure.*

The body is somewhat bean-shaped and is oval or round in cross-section. Seen from the ventral aspect—*i.e.* the side on which the flagella arise (fig. 1)—the anterior end appears flattened and is turned towards the left, where it terminates in an almost straight edge. This anterior portion of the organism is really an extension of the dorsal region, and is separated on the ventral surface from the main part of the body by a considerable depression, in which the mouth is situated. Fig. 2 shows a side-view and the relations of the anterior end to the depression, etc.

The trophonucleus is generally found towards the left side of the body when the organism is viewed from the ventral surface. It consists of a central deeply staining karyosome, which is connected with the nuclear membrane by means of strands which stretch across the extra-karyosomic zone, and at their insertion on the membrane produce slight thickenings.

The kinetonucleus is an irregular granular mass, often somewhat triangular in outline, and, on the whole, stains less intensely

than the karyosome of the trophonucleus. It has no nuclear membrane and is situated towards the ventral surface, close to the upper end of the mouth-depression. Its position can readily be made out in figs. 1 & 2.

The two flagella arise close to the anterior surface of the kinetonucleus from very indistinct blepharoplasts. The anterior flagellum is the shorter of the two, the posterior one being two or two and a half times its length. The organism ingests bacteria, which no doubt serve as a source of food, for the protoplast is often packed with cocci and other small forms of bacteria.

(b) *Reproduction.*

The first indication of approaching division which I have been able to find is the doubling of the anterior flagellum (fig. 4). At this stage no change is visible in the appearance of the nuclei. I am unable to say how the flagella become duplicated, but, judging from the fact that I have found no organisms showing protrusions like flagellar buds, and also that when the flagella become doubled the members of each pair are equal in length, I am inclined to the view that the original flagella split longitudinally.

The posterior flagellum becomes doubled later than the anterior one, and at this time the trophonucleus shows a marked change in appearance. The karyosome becomes much reduced in volume and divides into two equal parts, whilst at the same time the connecting strands between it and the nuclear membrane disappear. The nucleus elongates a little and stains rather more deeply, doubtless owing to the liberation of a chromatinic substance from the karyosome (figs. 5 & 6). Irregular granules of chromatin now appear at the periphery of the nucleus as the result of further fragmentation of the karyosome, and become concentrated towards the equatorial region. This particular stage is very difficult to determine, and I cannot say whether an equatorial plate is produced. So far as I can make out, there is merely an aggregation of chromatin granules on the surface of the nucleus in this region. These granules finally concentrate into four principal larger ones, which are arranged in two pairs. The stage figured on fig. 9 shows them apparently connected by two crossing strands. The nucleus now elongates, each end being somewhat pointed, and each pair of granules becomes drawn towards opposite ends of the nucleus. These stages are shown in figs. 10, 11, & 12. Soon after this a constriction appears in the middle of the nucleus, which now becomes rather dumb-bell-shaped (fig. 15). At about the same time, or even earlier, the four granules of chromatin begin to show signs of breaking down, and also stain less intensely (figs. 13–16). The constriction at the centre of the nucleus becomes carried still further, until two triangular daughter-nuclei are formed, each of which contains rather faintly staining chromatin granules. I have found it impossible to trace the later stages in the reorganisation of the daughter-nuclei, owing

to the fact that the flagellates are almost always crowded with deeply staining bacteria which obscure the nuclear elements. The next stage of the trophonucleus which I have certainly made out, is that in the daughter-organisms where the granules have become concentrated again into a central karyosome separated from the nuclear membrane by a clear zone (fig. 21). Whilst the above changes are proceeding, the kinetonucleus undergoes certain alterations. It increases in volume, and may either be triangular or rhomboidal in outline. One pair of flagella moves to either side of it, and at these points the kinetonucleus becomes somewhat drawn out. Soon after the formation of daughter-trophonuclei has taken place, the kinetonucleus elongates considerably, so that the pairs of flagella are carried farther apart. This elongation is carried on until a fairly long band of kinetonuclear material is produced, which finally separates into two portions—the daughter-kinetonuclei. I have not found the stages showing the constriction and division of the kinetonucleus, but there can be no doubt, I think, that the process is simple and direct.

Concurrently with the elongation of the kinetonucleus, the body of the flagellate becomes oval and then grows laterally, so that the longer axis of the body is that running from side to side, not antero-posteriorly. Division of the body is initiated by the formation of a constriction on the now shorter axis of the body, and becomes more and more pronounced until the two daughter-organisms are connected only by a short narrow strand of protoplasm. This finally breaks, and the two small organisms are produced. Division thus takes place along the antero-posterior axis of the body, and is therefore longitudinal.

(c) *Systematic Position.*

Because of the rapid spasmodic jumping motion exhibited by this organism in life, the name *saltans* is eminently applicable to it. From its general shape also, and the presence of two flagella, the posterior one being longer than the anterior, it easily fits into the genus *Bodo*. The difficult point to determine, however, is whether it should be classified as a *Bodo* or as a *Prowazekia*, for it possesses a kinetonucleus.

Alexeieff ('11 & '12) is of the opinion that all the species of *Bodo* possess a kinetonucleus, and holds that the genus *Prowazekia* is untenable. According to this author, my organism should go in the genus *Bodo*. I am not convinced, however, that his assertion concerning the presence of a kinetonucleus in *Bodo* is correct, for I have obtained a bimastigote form having the anterior flagellum shorter than the posterior one in which there is certainly no kinetonucleus, and which undoubtedly belongs to the genus *Bodo*. I therefore propose to place my organism in the genus *Prowazekia*. At the same time, however, I insert the name *Bodo* in brackets, because I think this form is identical with *Bodo saltans*. Alexeieff

('11) gives the dimensions of *Bodo saltans* as 6–10 μ in length by 3–5 μ in breadth. My organism is much smaller than this, measuring from 5–6 μ in length by 2·5–4 μ in breadth. In this respect it comes nearest to *Bodo minimus* Klebs, which is from 4–5 μ in length and is considered to be one of the smallest flagellates. The latter form, according to its original describer, is changeable in shape and has a creeping movement. My organism is very constant in shape, and always moves in rapid jumps followed by intervals of rest. There can be no doubt, then, that its specific name should be *saltans*.

Alexeieff ('11, p. 508) says that, without a doubt, *Bodo saltans* is the same organism as *Prowazekia parva* described by Nägler ('10). The latter organism differs considerably from that described above in several respects. Its protoplast is labile and takes on a great variety of shapes, whereas my organism is constantly bean-shaped. The method of division in both the tropho- and kinetonucleus is quite different from that described above. There is no fragmentation of the karyosome of the trophonucleus with the ultimate formation of four chromatin granules or chromosomes, but merely an equal division of the karyosome by promitosis, a centriole and a centrodesmose taking part in the process. The kinetonucleus divides in a similar manner. It is obvious, therefore, that Nägler's organism is a species distinct from mine, and his name *Prowazekia parva* should be allowed to remain.

Among the descriptions of members of the genus *Prowazekia* by Hartmann & Chagas ('10), Nägler ('11), Mathis & Léger ('10), Withmore ('11), Martin ('13), and Bělař ('14), the division of the trophonucleus of *Prowazekia asiatica* by Withmore comes nearest to that which I have given in the foregoing account. His figs. 18 and 19 show the presence of four principal chromosomes in the dividing nucleus, which, however, differs considerably in its earlier division stages from those observed in my organism.

(2) Tetramitus spiralis, sp. n. (Pls. II., III., figs. 23–45.)

(a) *Structure.*

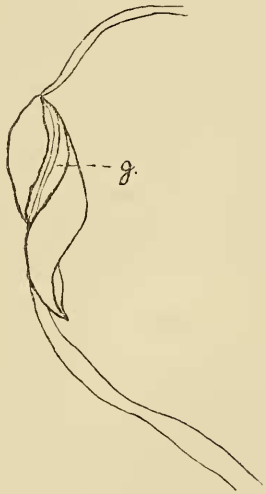
This interesting organism occurred in cultures from three different soils, viz.: Agdell 1867, Broadbalk 1865, and Barnfield 1870. I have been fortunate in obtaining it in large numbers, and have been able to make out most of the details of its structure and mode of division. I have not, however, observed it in the encysted condition, although I kept my cultures for a long time and had the organisms under close observation in hanging drops. I will first describe its structure and movements, and then deal with the process of reproduction.

In the majority of cases the body is pyriform in shape, having its extreme anterior end somewhat pointed. It may, however, become much elongated, and then appears more cylindrical.

A groove runs spirally from the anterior to the posterior end

of the body. Seen from the ventral aspect—*i. e.*, from the side on which the flagella arise (text-fig. 1)—the groove proceeds from right to left, and then curves round to the dorsal surface and reaches the posterior end of the body. If the body is of normal length there is only this one turn in the spiral, but if the organism is elongated there may be two turns. The accompanying text-figure, drawn from a specimen immediately after killing with osmic-acid vapour, shows the shape of the organism, the position of the groove, and the disposition of the flagella. The latter are arranged in two pairs—a shorter anterior pair and a longer posterior pair. The anterior pair is directed forwards during motion and the posterior pair is trailed backwards.

Text-figure 1.



Tetramitus spiralis.

Outline drawing as seen in ventral view, showing the relations of the groove (*g.*).

The members of each pair are frequently very closely applied, and often appear as one thick flagellum. When in active movement, which is caused by the lashing of the anterior pair of flagella, the organism progresses very rapidly and rotates on its long axis. The posterior pair of flagella appears to lie within the groove and extends beyond the posterior end of the organism, which swings from side to side of the line of motion owing to the rapidity of progression. The mouth is very difficult to locate, being ill-defined, but in certain examples I have made out its position a short distance from the anterior end as a depression in the groove. Bacteria are ingested and no

doubt serve as a source of food. There does not appear to be a contractile vacuole. The body measures from 8–12 μ in length.

Fixed and stained material shows that the protoplasm is alveolar in structure, and that the nucleus is situated at the anterior end of the body, as in *Trichomonas* and *Trichomastix* and similar forms. The nucleus is a vesicular structure of variable size, but often of quite large dimensions. There is no central karyosome, but the chromatin is disposed irregularly in two or more masses. Very frequently there are two semilunar blocks of chromatin situated on either side or on the anterior and posterior borders of the nucleus. It will be seen from the figures in what an irregular fashion the chromatin is arranged.

The flagella arise from blepharoplasts placed just anteriorly to the nucleus. They are four in number and, as stated above, are disposed in two pairs. They take their origin in four blepharoplasts, which when seen in side view (fig. 25) appear as two large granules in contact with each other, but when seen in face view are easily recognisable as being a group of four distinct granules in intimate contact (figs. 26 & 27). The posterior pair of granules is connected with the nucleus by means of two rhizoplasts, which appear as one short rod in fig. 25 but are well shown in the ventral view obtained in fig. 26. I made a very careful examination of this region, in order to determine if rhizoplasts were present connecting the anterior pair of blepharoplasts with the nucleus, and I am satisfied that the pair connecting the posterior blepharoplasts is the only one.

(b) *Reproduction.*

I will deal first with the multiplication of the flagella, because this always occurs prior to the division of the nucleus. The new flagella are produced by outgrowths from the body of the organism, and not by splitting of the old flagella. In this it resembles *Copromonas*, *Trichomonas*, and many other flagellates. The process is initiated by the growth in an anterior direction of each pair of blepharoplasts: a point very difficult to make out in many of the organisms, but well shown in other cases, one of which is represented in fig. 27. The flagellar buds arise as delicate hair-like outgrowths from the developing buds of the blepharoplasts. Each pair of flagella has thus a new pair of flagella produced immediately anterior to it. The new ones do not stain so deeply as the old flagella, as will be seen from the figures representing different stages of the division of the nucleus. In this way the original posterior pair acquires a new anterior pair, and the original anterior pair grows longer and becomes the posterior pair of one of the daughter-organisms, at the same time acquiring a new anterior pair. The four pairs of flagella thus produced gradually separate into two sets of two pairs, which finally come to take up positions at either end of the anterior face of the nucleus; but this migration takes place at different periods during the progress of nuclear

division. For example, fig. 28 shows an organism in which the flagella are widely separated, although the nucleus shows no sign of approaching division. I am unable to state the fate of the pair of rhizoplasts.

In the earliest stages in the division of the nucleus the chromatin seems to undergo some process of dissolution and reorganisation whereby certain parts of it, which stain less intensely than the rest, gradually come towards the centre of the nucleus. These take the form of roundish or irregular granules which, at the stage shown in fig. 31, appear to be arranged in a fairly regular manner on a kind of reticulum or network of linin threads. Whilst these changes are taking place, the remainder of the chromatin, having deeply staining properties, becomes arranged in the form of a ring round the periphery of the nucleus. This does not always happen in the very early stages, however, as is shown in fig. 33, where the deeply staining chromatin is still present as three blocks, whilst at the centre of the nucleus there is a group of lighter granules. The changes which occur in the lighter staining granules are very difficult to make out, but it seems as though they gradually concentrate towards the centre of the nucleus, and there become arranged in an irregular manner on a kind of plate. In figs. 32 and 33 there appear to be six principal granules disposed in two bands upon what seems to be a spindle formation. I do not wish, however, to lay stress on the presence of a spindle within the nucleus, for I have failed to make it out with any degree of distinctness, and even in those examples which present the spindle appearance, there are always irregularly-branching linin strands running in various directions, as shown in figs. 35 and 36.

In the stages represented in figs. 34–36, four principal round granules are present. These represent the nearest approach to chromosome formation in the whole series of changes. It would appear from these figures that the nucleus produces division centres from within, and is not dependent on the migration of the blepharoplasts to their antero-lateral positions for the formation of its poles of division. I have not been able to trace further the movements of the four internal chromosomes. In the succeeding stages the nucleus elongates somewhat laterally, and the peripheral ring of chromatin begins to break up and travel towards the lateral poles, at the same time advancing on to the dorsal and ventral surfaces of the nuclear membrane. In fig. 38 the connections between the blepharoplasts and the nucleus which ultimately become the rhizoplasts are fairly clearly seen. The lateral elongation of the nucleus now becomes more pronounced, and the chromatin, travelling along the linin threads of the nuclear network, becomes arranged in small granular masses towards the lateral poles. At this time the threads stretching across the centre of the nucleus can be made out fairly easily (figs. 39 & 40). These linin threads are doubtless absorbed, and two laterally situated daughter-nuclei are thus

produced. The chromatin now becomes re-arranged in granules of varying size and shape, as shown in figs. 41–44.

During the later stages of nuclear division, the anterior surface of the body becomes much drawn out and flattened. A depression now appears on this surface of the body and gradually travels backwards, and at the same time, in some cases, the protoplast extends laterally (fig. 44). In other cases the body becomes triangular in outline, and large vacuoles appear towards the centre of the body and, by rupturing, assist in the production of the daughter-organisms (figs. 42 & 43). Division of the body is thus longitudinal in direction. Fig. 45 shows two newly-formed organisms which have recently separated, their drawn-out tail-ends overlapping slightly.

(c) *Systematic Position.*

The possession of four flagella places my organism undoubtedly in the genus *Tetramitus* Perty, and though this to-day is a very mixed assemblage of forms, comprising, as it does, the free-living organisms described by Perty and by Klebs ('92), and also the parasitic forms *Tetramitus* (*Macrostoma*) *mesnili* (Wenyon, '10 *a*) and *Tetramitus* (*Macrostoma*) *caullery i* (Alexeieff, '11 *a*), there is no reason why I should create a fresh genus for its reception. As, however, I have been unable to discover any description or figures of any free-living member of the genus which fits my organism, I have decided to make a new species of it, namely, *spiralis*.

(3) Spironema multiciliatum Klebs. (Pl. III. figs. 46–48.)

(a) *Structure.*

This highly interesting organism occurred in one culture made from Broadbalk 1865 soil. It appeared both on the surface and at the bottom of the culture. My attention was first attracted to it by reason of its great length and its peculiar method of locomotion. It moved slowly in a very hesitating jerky manner for the most part, but would suddenly exhibit rapid and violent spiral twists commencing at its anterior end and travelling down the body, at which times it was propelled at a reasonably fast pace. It was obvious that the organellæ causing the slow jerky motion were situated at or towards the anterior end, though they could not be distinguished under a low power of the microscope. Towards the posterior end a contractile vacuole could be seen in diastole and systole.

I was able to obtain film preparations which, when fixed and stained, revealed the structure of the organism very clearly. The body is extremely long in comparison with the width, and is dorso-ventrally flattened. It measures anything from 20–50 μ in length, and averages about 4 μ in width. The middle region is generally the widest part of the body. The anterior end is either rounded or has a lateral knob-like portion on either side.

The posterior end is drawn out into a long and exceedingly fine tapering tail, and the contractile vacuole occurs just where the body begins to narrow down.

The flagella are numerous and comparatively short. They vary in number from seven to eighteen, and the smaller the organism the fewer the flagella. They are situated in most cases in two lateral rows towards the anterior end of the body, one row being dorsally and the other ventrally placed. I have carefully noted the disposition and number of the flagella, and find that they are not equally distributed on either side, but exhibit a considerable amount of variation in this respect. Klebs, on his Pl. xvi. fig 9 *c*, shows a row of flagella extending backwards on one side as far as the beginning of the tail. I have not found anything like this in my organisms.

A few of the organisms were fixed just as they were twisting spirally, and one of these is shown in fig. 48. It will be seen from this that the edges of the body are curved, and that the flagella have their origin close to the edges. Each flagellum arises from a small basal granule or blepharoplast distinctly seen in the stained material. I cannot say whether there is a mouth, and although I watched the creatures in life for a long time, I never saw them take in food. There are numerous large granular bodies, however, in the cytoplasm in many of the forms which appear to be ingested bacteria, and because of this, I am of the opinion that a mouth is present. I believe it is situated towards the anterior end, for I have made out, in some cases, a somewhat lighter area here which might be considered as the mouth. In the greater part of the body the cytoplasm is very finely granular and evenly distributed, but towards the posterior end, in the region of the contractile vacuole, it is frequently much vacuolated.

The nucleus is a very interesting structure. It is, in most cases, of considerable length, and is situated about half-way down the body. It consists of a long narrow rod of granular material, frequently one-quarter to one-third of the body in length. Towards the middle of it is placed a circular karyosome of deeply staining chromatin. The extra-karyosomic portion of the nucleus appears to be very little different from the general cytoplasm in staining reactions, and is separated from the latter on all sides by a very narrow clear space. There does not appear to be any nuclear membrane. At all events, I have not made out anything comparable with the nuclear membrane of other flagellates and amœbæ. From the appearance of the stained examples, it seems that all the chromatin is concentrated in the deeply staining karyosome, there being only small scattered granules in the rest of the nucleus.

The nearest approach to this nuclear apparatus which I have been able to find, is that which occurs in certain Euglenoidea, for example, in *Euglena viridis* (Keuten, '95), in which there is a fairly large nucleus consisting of a central karyosome

surrounded by a granular portion, the bulk of the chromatin being located in the karyosome, and the rest disposed in fairly large granules in the extra-karyosomic part. At first I was inclined to regard the extra-karyosomic portion of the nucleus as a macronucleus, and the karyosome as a micronucleus, taking the organism to be a ciliate. But the comparatively large size of the karyosome, and the fact that it occurs embedded in the centre of the rest of the nucleus, seems to rule out its micronuclear homology. Unfortunately, none of my preparations shows the organism dividing, so that I am unable to indicate the behaviour of the nucleus during these most important phases.

(b) *Systematic Position.*

After studying Klebs's ('92, p. 350) description and figures of the organism *Spironema multiciliatum*, there can be no doubt that my organism belongs to the same genus. It agrees in possessing about the same number of flagella, which are similarly situated, a posteriorly placed contractile vacuole, and in general appearance is the same. Klebs's organisms measured 14–18 μ in length by 2–3 μ in width, whilst none of my organisms is less than 20 μ in length, and they are about the same as his in width. This difference in length does not appear to me to be sufficiently important to warrant the creation of a new species for the reception of my organism. Klebs describes and figures two lateral spiral grooves commencing at the anterior end of the body and extending backwards as far as the beginning of the tail, and says that the flagella are inserted on the edge of each groove. These grooves correspond, I believe, to the dorsal and ventral surfaces of my organism when exhibiting its spiral twist, for there are really no true lateral grooves, the body being so thin. He made out nothing of the nuclear apparatus in his organisms, but from their appearance, and the number and disposition of the comparatively short flagella, he looked upon the creatures as probably forming a connecting-link between the Ciliata and Flagellata, as the following quotation shows:—"Ich halte es für sehr wahrscheinlich, dass diese vielgeisseligen Flagellaten einen Übergang zu den Ciliaten bilden und möchte speciell die Aufmerksamkeit auf diese noch so wenig bekannten Formen lenken."

From the appearance and structure of the nucleus, I think it is best to include *Spironema* in the Flagellata, though there is something to be said for Klebs's suggestion of it being a connecting-link between the Ciliata and Flagellata. The posterior position of the contractile vacuole is a ciliate characteristic, whilst the organellæ at the anterior end might equally be regarded as long cilia or short flagella. The forms which Klebs examined were obtained from ditch-water, so that there is nothing very remarkable in my obtaining the same organism in cultures of soil.

B. Rhizopoda.

Each of the soils yielded small amœbæ of the *limax* type, and I have been fortunate in obtaining a number of stages in the division of one or two of the forms.

(1) Amœba lawesiana, sp. n. (Pls. III., IV., figs. 49–65.)

I propose this name * for a small amœba which occurred in a culture of Broadbalk 1865 soil. I put up the culture in the hope of obtaining another kind of protozoon, but instead of getting this particular organism, I obtained an almost pure culture of the amœba in question. Unfortunately, I was unable to devote much attention at the time to observing the living organisms, and for this reason I am not able to state definitely whether a contractile vacuole is present or not. The conditions prevailing at the surface of the culture were very favourable to active life, for my permanent preparations show that the amœbæ were ingesting large numbers of bacteria and dividing forms are fairly abundant.

I have been fortunate in obtaining an almost complete series of dividing organisms, and from the appearance presented by the nucleus during these phases there can be no doubt that this amœba is very closely related to *Amœba glebæ*, which Dobell ('14) has recently described in great detail. It is also similar in its nuclear changes to *Amœba lamellipodia* (Gläser, '12), and the large amœba from liver-abscesses, described by Liston and Martin ('11), and also to *Amœba cucumis* and *Amœba gobanni-ensis* (Martin & Lewin, '14).

Nevertheless, it differs from all these in certain important details, which are dealt with later on, and for this reason I propose to create a fresh species for its reception.

It is rather smaller than *Amœba glebæ*, and the following are some of its principal measurements :—

Diameter of rounded forms	12–15 μ.
Diameter of nucleus	4– 5 μ.
Diameter of karyosome	2 μ.
Diameter of ripe cysts	10–11 μ.

(a) *Structure.*

When in motion, the body becomes extended in the typical *limax* shape and presents a blunt advancing pseudopodium. The protoplast is composed of an almost hyaline ectoplasm and a much vacuolated endoplasm. In fig. 49 the alveoli of the endoplasm are very irregular in shape and distribution, but in the almost spherical forms assumed during nuclear division the alveoli are fairly regularly distributed throughout the endoplasm and are more equal in size. The body is often crowded with ingested

* I have named this amœba after Sir John Lawes, the founder of the Rothamsted Experiments.

bacteria, and in those forms exhibiting the slug-like appearance the posterior end is frequently covered by an adherent mass of bacteria (fig. 49).

The nucleus consists of a large karyosome, which is separated from the nuclear membrane by a clear zone and an outer ring of faintly staining granules. The latter may apparently occur as very small discrete particles, as in fig. 49, or as a single ring of small blocks, as in fig. 50. I have not succeeded in making out any connecting strands between the karyosome and the nuclear membrane. It resembles the nuclei of *Amœba glebæ* and *Amœba lamellipodia* in possessing the ring of faintly staining granules.

(b) *Reproduction.*

The animal ceases to wander about and comes to rest, at the same time becoming spherical. I have not made out pseudopodia in any of these globular dividing forms. The earliest stage in the division of the nucleus which I have discovered is shown in fig. 50, where the karyosome has broken down into four principal masses. This fragmentation of the karyosome is continued until the central part of the nucleus originally occupied by the karyosome, or an area slightly larger than this, becomes filled with a mass which appears to be made up of very faintly staining particles, amongst which are lodged the rather more deeply staining granules produced by the disintegration of the karyosome. I cannot say whether the ring of faintly staining granules occurring in the "resting" nucleus takes any part in the division or whether they disappear. Dobell says that in *Amœba glebæ* they entirely disappear, and it may be the same in my organism. The fine particles produced by the fragmentation of the karyosome stain much less intensely than the original karyosome. They gradually aggregate and produce somewhat larger granules, which become connected up into a sort of chain formation, which lies in an irregular manner among the mass of linin particles. The nuclear membrane does not disappear, and does not seem to become any less distinct than during the "resting" condition of the nucleus.

The chain of chromatin granules or chromosomes, as they may perhaps be called, approaches the equatorial region of the nucleus, where it ultimately becomes disposed in the form of a ring (fig. 53). At this stage the first indications of a spindle make their appearance, becoming elaborated out of the linin matrix in which the ring of chromatin granules has been lying. The ends of the spindle are at first broad and rather flattened, but later on they become very sharply pointed. The plane in which the long axis of the spindle lies is slightly oblique to the horizontal plane of the nucleus. This is well seen in fig. 54, where the two ends of the spindle extend beyond the limits of the nuclear membrane which is represented in optical section, one end being over and the other under the nuclear membrane.

In the equatorial ring of chromatin granules I have not been able to distinguish at all clearly the separate constituent chromosomes. This may be due to the fact that they become very closely packed together. They are most distinctly seen in figs. 53 & 54. The equatorial ring becomes divided into two in the plane at right angles to the axis of the spindle. I have not discovered any organism showing the actual constriction of the chromosomes, but have obtained a stage where the two daughter-rings are very closely apposed (fig. 56). In the succeeding stages of division the two rings of chromatin gradually become separated from each other by a wider interval, owing to the elongation of the spindle, the fibres of which become quite distinct across the centre of the animal.

The poles of the spindle remain sharply pointed until a late stage in the separation of the new chromatin bands (figs. 59 & 60).

After the stage which is depicted in fig. 55, the word "band" more accurately describes the appearance presented by the daughter chromatin elements, for I have not been able to make out any ring-like structure after carefully focussing on these parts. Neither have I been able to distinguish separate chromosomes, for each band appears to be composed of numerous fine granules. The nuclear membrane appears to remain intact up to the stage shown in fig. 56, after which, however, it is not distinguishable, and I suppose it disappears entirely.

The animal now elongates in the direction of the long axis of the spindle and becomes ellipsoidal in outline (fig. 59). This figure shows an interesting condition of the spindle-fibres between the chromatin bands, in that a twist in them seems to have been produced as though one of the bands had rotated through an angle of 180°. A constriction now appears round the animal, and the first stage in the process of fission is brought about. Fig. 60 represents this stage, and it is easily seen that the poles of the spindle are sharply pointed at this time.

The process of the re-formation of the daughter-nuclei now begins. The pointed poles of the spindle disappear, and the chromatin granules become scattered irregularly in a mass of faintly staining linin particles which are apparently formed by the break-up of the outer portion of the spindle-fibres. In fig. 61 one daughter-nucleus is seen to consist of a crescentic area of linin particles in which the small granules of chromatin are scattered, whilst at the other end of the spindle the daughter-nucleus consists of a small though well-developed central granule, no doubt formed by a fusion of smaller granules, surrounded by a ring of linin particles, from which it is separated by a clear zone. I think there can be no doubt that the crescentic daughter-nucleus represents an earlier stage in the process of reorganisation than the round form in the other part of the constricted amœba.

In fig. 62 the constriction of the parent amœba has been carried a little further, and the spindle-fibres between the

re-forming nuclei could be made out on focussing very carefully. It can be seen from this figure that the reorganisation process takes place earlier on the outer side of each nucleus than on the inner side. I have not been able to distinguish any reticulate arrangement in the linin particles which are laid down as the process of reorganisation commences. The fission of the body is now carried a little further, and the two daughter-organisms are produced. There does not appear to be any connecting strand of protoplasm between the two products of fission, though in all these stages this region is extremely difficult to make out, owing to the presence of large masses of adherent bacteria, which I have purposely omitted from the drawings.

The new karyosome now increases in size by the absorption of the remaining fine granules of chromatin. It is no longer possible to distinguish any spindle-fibres, and each nucleus becomes rounded off. The new nuclear membrane is apparently formed from the zone of linin surrounding the new karyosomes, and from this zone also the peripheral ring of feebly staining granules is also produced. The only difference between the nucleus of the stage represented in fig. 63 and that of a full-grown animal is merely one of size.

(c) *Encystation.*

On the same preparations which showed dividing animals, I obtained a few stages revealing the process of encystation. The first indication of this is the production of intensely staining small round granules in the endoplasm, as shown in fig. 64. In this animal I could discover very few ingested bacteria, and it is evident that the normal process of digestion becomes suspended with the beginning of encystation. There is practically no difference in the appearance of the nucleus during the process of encystation, and even when the cyst-membrane has become well defined, as in fig. 65, it was still possible to distinguish all the principal structures of the nucleus. The karyosome in the encysting animals is rather smaller than in normal active forms. As encystation proceeds, there is a gradual contraction of the endoplasm round the nucleus, so that the ectoplasm is left as a distinct region free from granules. This is particularly well shown in fig. 64, where the line of separation between the two constituents of the protoplast is especially marked. The animal diminishes somewhat in bulk, and the cyst-membrane is laid down around it. This later on becomes much corrugated and indented, as shown in fig. 65. It is quite well defined at this stage, but becomes somewhat thicker at a later period; a point which I have determined by the examination of empty cysts. There does not appear to be an endocyst. In possessing deeply stainable granules, the cysts differ from those of *Amœba glebæ*, in which Dobell describes non-stainable extremely refractile granules. I do not know what the real nature of the granules

produced in the endoplasm is, but they are of fairly common occurrence in the cysts of other forms of *limax* amœbæ. At all events they are not particles of chromatin extruded from the karyosome, for this does not diminish in bulk to any great extent, and, moreover, there is a sufficient volume of granular material produced in the endoplasm to make several karyosomes if it were fused together. Probably they are of a reserve food character. I cannot, however, throw any light on their presence or absence in newly excysted organisms, for I did not make any observations on the excystation of this amœba.

(d) *Remarks.*

Dobell has gone very thoroughly into the differences and similarities between his *Amœba glebæ* and its nearly related forms, so that it is quite unnecessary for me to go into this question in detail. I will merely point out, therefore, in what respects my organism differs from or resembles *Amœba glebæ*.

It is obvious, from a comparison of the figures illustrating this account and that of Dobell, that the amœbæ to which they refer are very closely related in their method of nuclear division. The type of division is the same in each, and it is merely in details that differences are presented. The most important are the following:—

1. *Amœba lawesiana* is a somewhat smaller organism than *Amœba glebæ*.
2. The nuclear membrane persists to a much later stage of division in *Amœba lawesiana* than in *Amœba glebæ*.
3. The spindle formed in the division of the nucleus is sharply pointed at each end in *Amœba lawesiana* and is rounded or barrel-shaped in *Amœba glebæ*.
4. The resting-cyst of *Amœba lawesiana* is irregular in outline, whereas that of *Amœba glebæ* is perfectly round and has a smooth outer wall.
5. Within the endoplasm of the cyst of *Amœba lawesiana* large numbers of deeply staining granules are produced, whereas in the cyst of *Amœba glebæ* highly refractive granules occur.

(2) Amœba agricola, sp. n. (Pl. IV. figs. 66–74.)

I propose to describe under this specific name a small amœba which occurred in one of the cultures made from Hoosfield 1868 soil. It exhibits some rather remarkable appearances during the division of the nucleus, which seem to differ from any of the already described nuclear divisions in amœbæ; and it is on the strength of this fact that I propose the creation of a new species for its reception. I made no special observations on the live animals, so that, in this respect, what I have to say about them is, unfortunately, incomplete. My notes merely record the presence of numerous *limax* amœbæ in this particular culture.

I made one or two film preparations, which, after fixation and staining, showed the presence of large numbers of amœbæ, together with the flagellate *Cercomonas longicauda*. In going carefully over one of these preparations, I discovered a number of interesting stages of dividing nuclei; and though I have not obtained a very complete series of these, I have made out sufficient to show that I am dealing with an organism hitherto undescribed.

(a) *Structure.*

There is nothing remarkable in the appearance of the ordinary individuals. The body presents an endless variety of shapes, and the pseudopodia are very irregular and lobose, whilst the distinction between ectoplasm and endoplasm is not at all clear. So far as I can ascertain, the endoplasm is not alveolate in structure. At any rate, if alveoli are present they are not large and distinct like those in *Amœba lawesiana* and *Amœba glebæ*, for in only one of the animals could I make out anything at all approaching alveoli. I do not wish to emphasize this point, however, for the preparation was slightly over-differentiated and the cytoplasm in all the amœbæ was only very faintly stained. On the whole, the endoplasm appears very finely granular in structure, with somewhat denser masses scattered about in it; and the figures which illustrate this account represent it fairly accurately.

The resting nucleus consists of a central deeply staining karyosome, separated by a clear zone from the nuclear membrane, with which it appears to be connected by very feebly staining strands stretching across the zone at various points. I have not been able to make out the presence of a ring of granules just within the nuclear membrane as in *Amœba lawesiana*. The principal measurements are as follows:—

Length of body 12–15 μ, though this measurement is not of much value because of the very irregular shape of the organism.

Diameter of nucleus, 2·75–3 μ.

Diameter of karyosome, 1·8–2 μ.

It is thus rather smaller than *Amœba lawesiana*.

(b) *Reproduction.*

The body does not become globular during nuclear division as in *Amœba glebæ* and *Amœba lawesiana*, but retains its very irregular appearance. The karyosome in the earliest stages of division loses its rounded shape and increases in size. At the same time it begins to break up into a number of ill-defined granules, which appear to rest on a matrix which stains only feebly. I have only encountered a few of these early stages, and therefore cannot give much information concerning the changes which go on at this period.

The final result of the break-up of the karyosome is the

formation of irregular chromosomes, some of which appear rod-like and others rather rounded. While these changes are taking place the whole nucleus increases in size and becomes barrel-shaped, attaining a length of 6–7 μ. A few spindle-fibres make their appearance within the nucleus, but no definite spindle comparable with that found in *Amœba lawesiana* is produced. Moreover, the fibres seem to lie on the nuclear membrane rather than within the cavity of the nucleus. The chromosomes now become arranged on the fibres, but I have not discovered any examples which show all the chromosomes arranged in an equatorial ring or band. All the stages of this phase of division show two principal groups of chromatin granules or chromosomes at each end of the long axis and other chromosomes irregularly disposed in the equatorial region. In the latter region the individual chromosomes are extremely difficult to distinguish, and it is therefore practically impossible to count them. There appear, however, to be about eight chromosomes or chromatin masses produced within each nucleus, four of which travel to each end. A description of these stages of division is very difficult to make owing to the fact that no two stages exactly agree, as will be seen on referring to figs. 68–70. All of them are, however, of the same general type, and a detailed description of each is unnecessary. The chromosomes ultimately become drawn to the two poles of the nucleus, a stage which is well shown in fig. 71.

Shortly after this, or even earlier, the chromosomes begin to break up and lose their distinct outline. As a result of this, a granular mass of chromatin, rather triangular in outline, is produced at each end of the nucleus (figs. 72 & 73). The nucleus now begins to elongate, and the chromatin is reorganised into daughter-nuclei, which thus gradually separate further and further apart. In fig. 73 a stage is shown in which a dumb-bell appearance is presented by the two rounded daughter-nuclei and the nuclear membrane constricted between them. This is the latest stage of division that I have obtained. I have failed to discover any stages showing fission of the animal, and it seems to be fairly evident that this occurs after nuclear division is quite complete. In this connection it is interesting to note that I have found a large number of bi-nucleate amœbæ on the same preparation. It is possible that these are forms in which fission is retarded, or again they may be abnormal individuals, for I have found one or two tri-nucleate forms as well.

(c) *Remarks.*

The nuclear division in *Amœba agricola* differs from that which occurs in any other amœba. It is obviously a modified mitosis, but it is not easy to connect it up with any of the numerous mitotic nuclear divisions which have been described and figured in other amœbæ.

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EXPLANATION OF THE PLATES.

All the figures are camera lucida drawings, and were made with the aid of Zeiss 2 mm. apochromatic objective and compensating oculars 18 and 12, giving approximate magnifications of 2786 and 1833 diameters respectively.

Plate I.

All figures magnified 2786 diameters approximately.

Prowazekia (*Bodo*) *saltans*.

Fig. 1. Organism seen from the ventral aspect.
2. Side view.
3. Dorsal view, a small blepharoplast at the base of each flagellum.
4. Doubling of the anterior flagellum.

Figs. 5–8. Early stages in nuclear division.
9–12. Four small chromosomes present in the nucleus.
13–16. Fragmentation of chromosomes and division of the nucleus.
17–20. Elongation and division of kinetonucleus and constriction of the body into two daughter-organisms. The new trophonuclei are unrecognisable in these stages.
Fig. 21. Two daughter-organisms nearly separated; new trophonuclei visible.
22. Small recently separated daughter-form.

Plate II.

Owing to the exigencies of space in making up the Plate, the full extent of the two pairs of flagella is shown only in figs. 23 & 26.

Tetramitus spiralis, sp. n. All figures × 2786.

Figs. 23 & 24. Two normal forms, showing the groove.
25 & 26. Showing the relations of flagella, blepharoplasts, rhizoplasts, and nucleus.
Fig. 27. Showing new flagella arising from anteriorly enlarged blepharoplasts.
28. The flagella have migrated before the nucleus shows signs of division.
Figs. 29–33. Successive stages in early phases of nuclear division.
34–36. Stages showing four principal chromatin masses within the dividing nucleus.
37–41. Later stages in nuclear division.

Plate III.

Figs. 42–45. *Tetramitus spiralis*, sp. n. × 2786.

Figs. 42–44. Later stages of division, showing the formation of daughter-organisms. In fig. 43 there appears to be a production of large vacuoles on the longitudinal axis of the body.
Fig. 45. The two new organisms have just separated.

Figs. 46–48. *Spironema multiciliatum*. × 1833.

Fig. 46. A rather small form having 8 flagella and showing the contractile vacuole at the beginning of the tail. The extra-karyosomic part of the nucleus has very small granules on its outer edge.
47. A longer form showing 18 flagella, rather irregularly disposed.
48. A long form showing spiral twist of the anterior part of the body and contractile vacuole towards posterior end.

Figs. 49–55. *Amœba lawesiana*, sp. n. × 1833.

Fig. 49. Normal individual of typical *limax* form, showing feebly staining granules just inside the nuclear membrane, and a mass of adherent bacteria at posterior end.
Figs. 50–52. Early stages in nuclear division showing disintegration of the karyosome and the production of a chain of chromatin granules or chromosomes.
Fig. 53. The formation of the spindle at first, having broad ends extending beyond the nuclear membrane The chromosomes are arranged in an equatorial band.
Figs. 54 & 55. The spindle has become pointed at each pole and is placed obliquely to the horizontal plane; ring of chromosomes.

Plate IV.

Figs. 56–65. *Amœba lawesiana*. × 1833.

Fig. 56. Equatorial ring just divided.
Figs. 57 & 58. Elongation of the spindle and separation of the new chromatin bands.
Fig. 59. Late stage of nuclear division, showing a twisting of the central portion of the spindle.
60. Commencement of constriction of the organism.

Figs. 61–63. Completion of fission and reorganisation of the daughter-nuclei.
64 & 65. Stages in encystation showing the production of deeply staining granules within the endoplasm. In fig. 64 the endoplasm appears sharply separated from the ectoplasm.

Figs. 66–74. *Amœba agricola*, sp. n. × 2786.

Fig. 66. Normal form.
67. Granulation of the karyosome.
Figs. 68–70. Showing a variety of stages in the arrangement of the chromosomes on the nuclear spindle. In each case there appear to be two principal chromosomes at each pole and variously situated chromosomes in the equatorial region.
Fig. 71. The chromosomes are drawn to each pole.
Figs. 72–74. Granulation of chromosomes and formation of daughter-nuclei.